

## Problems related to the use of sodium dodecylsulfate in high-performance liquid chromatography

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Sodium dodecylsulfate (sodium laurylsulfate) (SDS) is being used increasingly as a modifier in aqueous buffer mobile phases for the size-exclusion chromatography of proteins on siliceous supports<sup>1,2</sup>. SDS is a widely studied surfactant, and surface chemists have become aware of the variation in properties of commercial preparations, primarily due to impurities. The major impurities may be shorter and longer chain homologues, unreacted alcohols and electrolytes. It has been shown<sup>3</sup> that price and stated purity are not reliable indicators of the product's integrity. When SDS-potassium phosphate buffers were used as mobile phases for high-performance liquid chromatography (HPLC) in our laboratory, instability of siliceous size-exclusion columns was encountered. Sometimes when the mobile phase concentrations were changed, cloudy effluents were observed. In the course of studying these factors, we have made some observations about the properties of SDS-containing phosphate buffers which may be relevant to their use in chromatography.

### EXPERIMENTAL

Potassium phosphate buffers were prepared by mixing 150 ml of 0.1 M  $\text{KH}_2\text{PO}_4$ , 123 ml of 0.1 M NaOH (both ACS Reagent Grade), 30 g of SDS and 0.6 g of sodium azide, and diluting to 3 l with deionized, distilled water. When necessary small volumes of acid or base were added to adjust the pH to 7.5. As the buffer was intended for use in HPLC, it was filtered through a bacteriological filter (pore size 0.45  $\mu\text{m}$ ).

Four sources of SDS, listed below in expected order of increasing purity, were used. A was a commercial laboratory-grade material, B a commercial product labeled 98% pure, C a commercial sample labeled electrophoresis purity reagent and D a sample of SDS synthesized and purified in-house.

A potassium phosphate buffer containing no SDS was prepared as above and was added to the SDS-potassium phosphate buffers in order to study SDS concentration effects. Salt ion concentration effects were examined by adding 0.015 M NaCl and 0.015 M KCl to SDS-potassium phosphate buffers. Sodium dihydrogen phosphate buffers with and without SDS were prepared by mixing 150 ml of 0.1 M  $\text{NaH}_2\text{PO}_4$  in place of 0.1 M  $\text{KH}_2\text{PO}_4$ , and a separate series of parallel experiments was conducted.

## RESULTS AND DISCUSSION

Table I shows the effect of addition of potassium phosphate buffer to SDS-potassium phosphate buffer. A precipitate resulted when 34.7 mM (1%) solutions of SDS from all four sources were diluted, although the concentration at which precipitation started was different in each instance and visual estimates of the amount of precipitate varied. Large amounts of amorphous precipitates occurred with SDS samples of lower purity. Lesser amounts of transparent, crystalline precipitates occurred with SDS samples C and D. It is conceivable that as the SDS concentration becomes less than the critical micellar concentration ( $2-8 \text{ mM}$ )<sup>3</sup>, impurities which are likely to be solubilized in the micelle become apparent as precipitates due to the reduction in number of micelles.

TABLE I

### PRECIPITABILITY OF SDS AFTER ADDITION OF PHOSPHATE BUFFER

+ denotes precipitate, ++ denotes heavy precipitate and — denotes no precipitate.

SDS sample	Final SDS concentration (mM) *						
	31.2	26.0	22.9	17.3	11.5	8.7	3.5
A	—	—	+	+	+	++	+
B	—	—	—	—	+	++	+
C	—	—	—	—	+	+	+
D	—	—	—	—	—	+	+

\* The concentration of SDS after potassium phosphate buffer containing 1% SDS and potassium phosphate buffer without SDS were mixed.

Solutions of KCl and of NaCl were added to buffers prepared with SDS samples A, B, and C. As can be seen in Table II, additions of KCl caused precipitation in buffers whereas additions of NaCl did not. When SDS buffers were prepared with  $\text{NaH}_2\text{PO}_4$  instead of  $\text{KH}_2\text{PO}_4$ , no precipitates were observed when an experiment parallel to that summarized in Table I was repeated. Potassium ion has been observed in some instances to be a better coagulating ion for negative suspensions<sup>4</sup>.

TABLE II

### PRECIPITABILITY OF SDS AFTER ADDITION OF KCl AND NaCl SOLUTIONS

Symbols as in Table I.

SDS sample	Final SDS concentration (mM) *			
	17.3	11.5	8.7	3.5
A ( $\text{K}^+$ )	+	++	++	++
A ( $\text{Na}^+$ )	—	—	—	—
B ( $\text{K}^+$ )	—	+	++	+
B ( $\text{Na}^+$ )	—	—	—	—
C ( $\text{K}^+$ )	—	—	+	+
C ( $\text{Na}^+$ )	—	—	—	—

\* The concentration of SDS after potassium phosphate containing 1% SDS and 0.015 M aqueous salt solutions were mixed.

It is possible that the surface structure and functionality of the chromatographic support and the micro-environment at the surface could influence the effects reported above. A lowering of the critical micelle concentration at the surface of cation exchangers has been reported<sup>5</sup>. Thus, precipitation might occur as chromatography progresses as well as when conditions are changed. We have observed increased pressure drops when SDS-potassium phosphate buffers were used in HPLC. When the columns were unpacked, foamy precipitates were found. Work is continuing to determine factors that influence the column life, but at this stage the following suggestions can be made regarding the use of SDS buffers in chromatography: (1) SDS of the highest purity should be used and (2) sodium phosphate-SDS buffers, rather than potassium phosphate-SDS buffers, should be used, as they are more stable.

#### REFERENCES

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